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SOIL CONDITIONS AND ROOT DISEASES

VIII. Rhizosphere Microflora of Some of the Important Crop Plants of South India

By V. Agnihothrudu

(*University Botany Laboratory, Madras*)

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(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

In recent years considerable attention has been paid to the study of the "rhizosphere" of crop plants, particularly in relation to soil-borne diseases of plants (Timonin, 1940, 1941, 1947; Lochhead, *et al.*, 1940; Hildebrand and West, 1941; West and Hildebrand, 1941; Harper, 1951). The results of investigations so far reported on this subject indicate a fruitful line of work and appeared to be important. During the past few years the problem of soil conditions in relation to root diseases has received attention from workers in this laboratory and as a part of this general programme of research, the study of the rhizosphere microflora of certain crop plants was undertaken, being a preliminary to a better understanding of the problems of infection, disease resistance, etc. The results of the study are presented in this paper.

Materials and Methods

Infected soil from the laboratory garden plot was passed through a 1/16" mesh sieve and filled into earthenware pots. Twenty seeds per pot were sown. A series of unplanted pots served as control. At each sampling 10–20 plants were taken from each series for rhizosphere analysis. The block of soil surrounding the plant roots was carefully cut out, gently crushed with as little of tearing of roots as possible. The superfluous soil was removed by gently tapping the root system. The tops of the seedlings were cut off and roots with the soil attached deposited into a sterile Erlenmeyer flask with 100 ml. of sterile tap water and suitable dilutions were prepared. In fully grown plants only the representative portions of the root system were selected (Timonin, 1940). The first dilution was shaken by hand for 5 minutes and the subsequent dilutions 1 minute each which were prepared on the basis of 1 : 10, *i.e.*, each higher dilution made by taking 10 ml. of lower dilution and adding to 90 ml. of sterile water. The numbers of micro-organisms were estimated by plating method (Brierley, *et al.*, 1927).

Counts were made on 4 replicate plates and average counts recorded. Modified Horne and Mitter's medium (pH_{4-5}) was used for fungi, and for bacteria and actinomycetes Thornton's medium (pH_{6-8}). Fungal counts were taken on the 5th and the 7th days, bacteria on the 7th and the 10th days, actinomycetes after 11 days of incubation at laboratory temperature (29–31° C.). The dilutions of control soil were made on 10 g. basis.

The weight of the rhizosphere soil was determined by removing the roots from the original flask and draining off its contents completely into a previously weighed Petri dish. The water was evaporated on a water-bath and the soil residue dried to constant weight in an oven at 105° C. The weight of the soil lost in the first dilution from the original flask was determined by evaporating 10 ml. of the first dilution on a watch-glass and this was added to the weight of the rhizosphere soil every time. From this final weight of the rhizosphere soil the dilution coefficient was computed. The numbers of fungi, bacteria and actinomycetes are expressed on the basis of 1 g. moisture-free soil. All the results were statistically analysed.

EXPERIMENTAL

Representative plants from four different families, namely, Leguminoseæ: pigeon-pea (*Cajanus cajan*); sann-hemp (*Crotalaria juncea*) ; cluster bean (*Cyamopsis tetragonoloba*); french bean (*Phaseolus vulgaris*); Pedalineæ: sesame (*Sesamum indicum*); Malvaceæ: K.2 cotton (*Gossypium arboreum*, v. *neglectum* forma *indica*) and Gramineæ: sorghum (*Sorghum dochna*, v. *irungu*) were selected for rhizosphere studies which extended over a period of three months. All seeds were sown after surface sterilization with 1: 1000 aqueous mercuric chloride. Cotton seeds were delinted with concentrated sulphuric acid. Sampling for rhizosphere analysis was done at 15, 31, 46, 61, 77 and 93 days after complete germination. Of the plants studied except pigeon-pea, cotton and *Sorghum*, in the remaining flowering and pod-setting occurred during the estimations. In french bean and cluster bean flowering was observed about 30 days after germination whereas in sesame and sann-hemp it was recorded after 40 and 62 days respectively.

RESULTS

Quantitative Nature of the Rhizosphere Microflora

1. Significantly higher numbers of bacteria, fungi and actinomycetes were recorded in the rhizosphere than in the control soil. A decreasing order of ' rhizosphere effect ', *i.e.*, the rhizosphere: control soil ratio was noted as follows: bacteria > fungi > actinomycetes (Tables I, II and III; Text-Fig. 1).

TABLE I

Numbers of Fungi, Bacteria and Actinomycetes in the Rhizosphere

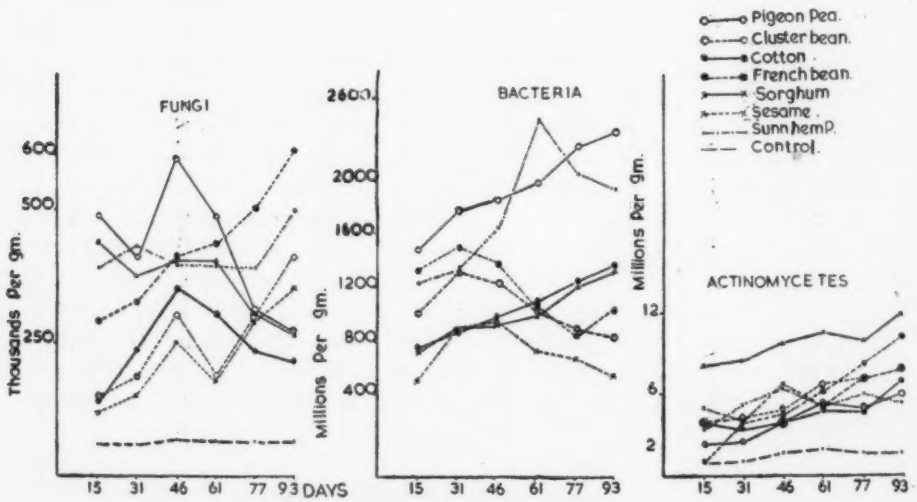
Plants	Fungi*						Bacteria*						Actinomycetes*					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	61	77	93
Pigeon-pea ..	488	415	575	475	305	280	1435	1783	1873	1993	2230	2380	2·3	2·5	4·0	5·5	5·0	6·0
Cluster bean ..	145	175	295	190	298	405	998	1315	1233	1040	900	830	4·0	4·3	5·0	6·8	7·0	8·0
Cotton ..	130	225	350	303	230	223	743	833	998	1058	1250	1350	4·0	3·5	4·0	5·0	5·0	7·3
Sorghum ..	440	370	413	400	308	270	720	858	903	1003	1230	1345	8·5	8·8	9·8	10·8	10·0	12·0
Sesame ..	118	150	248	180	283	353	493	835	943	723	693	553	5·0	4·0	6·8	5·3	7·0	8·0
French bean ..	288	315	410	433	493	595	1320	1475	1390	1048	883	1033	1·5	3·8	4·5	6·3	8·3	10·3
Sann-hemp ..	385	430	395	233	380	490	1248	1370	1670	2450	2040	1930	3·5	5·0	6·5	5·3	6·0	5·5
Control soil ..	55	55	65	60	60	60	7·0	7·0	10·0	9·0	10·0	10·0	1·5	1·3	1·8	2·0	1·8	1·8

* Fungi in thousands, Bacteria and Actinomycetes in millions per 1 g. moisture-free soil.

TABLE II

Rhizosphere : Soil Ratios (Rhizosphere Effect)

Plants	Bacteria		Fungi		Actinomycetes	
	Age of the plant in days 15	93	Age of the plant in days 15	93	Age of the plant in days 15	93
Pigeon pea ..	205	236	8·9	4·6	1·5	3·8
Cluster bean ..	142	83	2·6	6·7	2·6	4·4
Cotton ..	106	135	2·4	3·7	2·6	4·0
Sorghum ..	102	135	8·0	4·5	5·6	6·6
Sesame ..	70	56	2·2	5·9	3·3	4·9
French bean ..	188	104	5·2	9·9	1·0	5·6
Sann-hemp ..	178	193	7·0	8·1	2·3	3·0



TEXT-FIG. 1. Showing the fungal, bacterial and actinomycete numbers during the six rhizosphere analyses.

2. In general legumes had greater numbers of fungi and bacteria in the rhizosphere than the rest of the plants and *Sorghum* had the highest numbers of fungi among the non-legumes (Tables I, II and III; Text-Fig. 1).

TABLE III

Table of Significance

Age of plant in days	Fungi	Bacteria	Actinomycetes
15	Pp, Sor, Sh, Fb, Cb, Cot, Ses, C	Pp, Fb, Sh, Cb, Cot, Sor, Ses, C	Sor, Ses, Cb, Cot, Sh, Pp, Fb, C
31	Sh, Pp, Sor, Fb, Cot, Cb, Ses, C	Pp, Fb, Sh, Cb, Sor, Cot, Ses, C	Sor, Sh, Cb, Ses, Fb, Cot, Pp, C
46	Pp, Sor, Fb, Sh, Cot, Cb, Ses, C	Pp, Sh, Fb, Cb, Cot, Ses, Sor, C	Sor, Ses, Sh, Cb, Fb, Pp. Cot, C
61	Pp, Fb, Sor, Cot, Sh, Cb, Ses, C	Sh, Pp, Cot, Fb, Cb, Sor, Ses, C	Sor, Cb, Fb, Pp, Sh, Ses, Cot, C
77	Fb, Sh, Sor, Pp, Cb, Ses, Cot, C	Pp, Sh, Cot, Sor. Cb, Fb, Ses, C	Sor, Fb, Cb, Ses, Sh, Pp, Cot, C
93	Fb, Sh, Cb, Ses, Pp, Sor, Cot, C	Pp, Sh, Cot, Sor, Fb, Cb, Ses, C	Sor, Fb, Ses, Cb, Cot, Pp, Sh, C

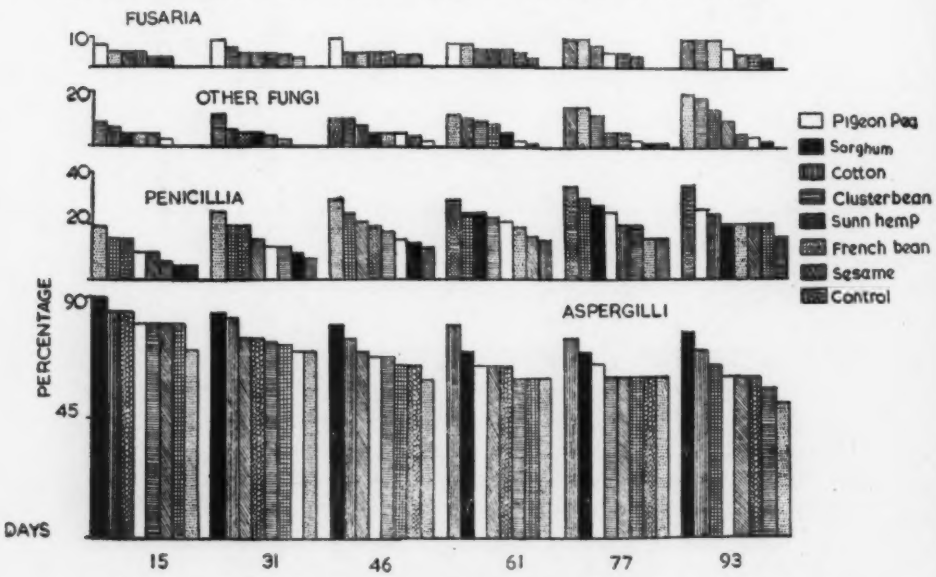
Pp = Pigeon pea
Sor = Sorghum
Sh = Sann-hemp
Fb = French bean
Cb = Cluster bean
Cot = Cotton
Ses = Sesame
C = Control soil

3. A decrease in fungal numbers was recorded with advance in age of the plants but in flowered plants (french bean, cluster bean, sesame and sann-hemp) an increase in numbers was recorded after flowering. In the unflowered plants the bacteria increased in numbers with the age of the plant but in the flowered plants this increase continued up to the time of flowering only followed by a fall in the pod setting stages of the plants (Tables I and III; Text-Fig. 1).

4. *Sorghum* had consistently higher numbers of actinomycetes in the rhizosphere than in the case of the other plants studied. Although in the seedling stages no significant differences between rhizosphere and control soil numbers of actinomycetes were seen, with the advance in age of the plants the stimulative effect of the root was evident (Tables I and III; Text-Fig. 1).

Qualitative Nature of the Rhizosphere Fungal Flora

(*a*) *Fungi in the Dilution Plates.*—In order to make a critical study of the nature and abundance of various fungi occurring in the rhizosphere of different plants, the fungal colonies in the dilution plates were carefully examined and identified to the genus. The approximate numbers and percentage occurrence of some of the more predominant soil fungi, namely, *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., were calculated from the dilution plates and the data presented in Table I, Text-Fig. 2.



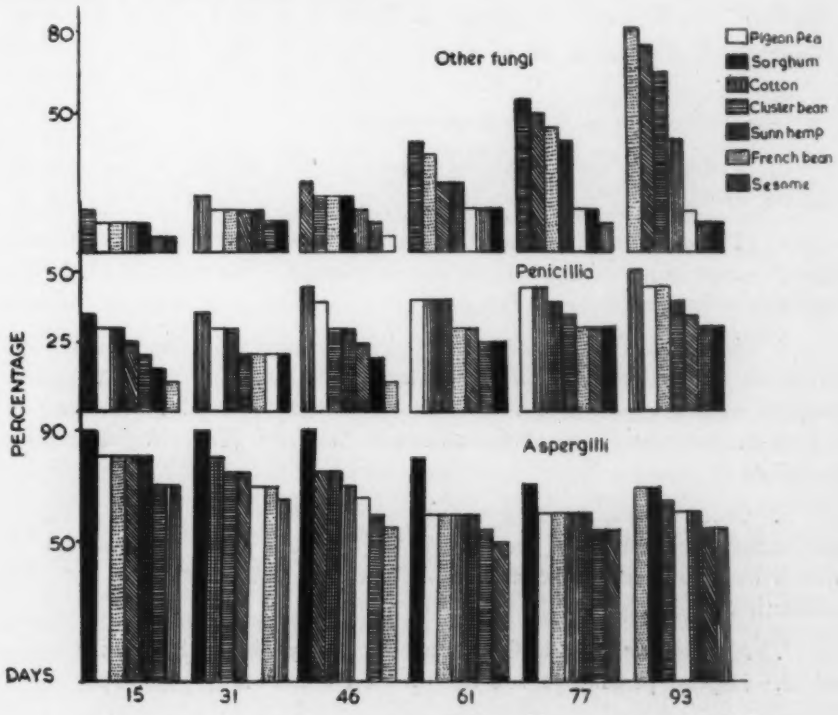
TEXT-FIG. 2. Showing the percentage occurrence of Aspergilli, Penicillia, Fusaria and other fungi in the dilution plates.

(*b*) *Fungi from Root Plantings.*—The roots of the plants used in the dilution were removed, washed in several changes of sterile tap water and plated on modified Horne and Mitter's medium. The percentage occurrence of various fungi was recorded and expressed on twenty root-bit basis (Text-Figs. 3 and 4).

Conclusions

1. *Aspergilli.*—This is by far the most predominant fungus in the rhizosphere and highest percentage was recorded in *Sorghum* and cotton (Table IV; Text-Figs. 2 and 3).

2. In general both in the control and rhizosphere soils, decrease in numbers and percentage occurrence of Aspergilli was recorded towards the final estimation except in flowered plants where an increase at the end was registered (Table IV; Text-Fig. 2).



Text-Fig. 3. Showing the percentage occurrence of Aspergilli, Penicillia and other fungi in the root platings.

TABLE IV

Numbers of Aspergilli, Penicillia and Other Fungi in the Rhizosphere

Plants	Aspergilli						Penicillia						Other Fungi					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	51	77	93
Pigeon pea ..	390	294	398	312	201	167	50	63	106	120	87	74	10	21	13	5	2	20
Cluster bean ..	120	133	204	114	180	220	12	21	54	45	60	60	13	22	30	20	36	76
Cotton ..	204	263	263	240	173	154	67	42	42	45	46	55	8	0	27	0	0	3
Sorghum ..	396	315	328	280	217	210	22	37	62	100	87	54	22	18	23	20	4	5
Sesame ..	96	113	175	117	168	210	12	24	55	32	42	70	4	6	10	20	42	35
French bean ..	203	224	246	258	300	300	58	80	123	86	75	120	15	2	20	51	75	120
Sann-hemp ..	312	314	260	138	228	308	58	86	100	58	114	98	0	0	20	20	19	30
Control soil ..	47	41	39	36	36	36	8	11	13	18	21	21	0	0	8	0	3	3

Fungi in thousands per 1 g. of moisture-free soil.

3. Unlike Aspergilli, Penicillia show a gradual increase in the numbers and percentage with the advance in age of the plant (Table IV; Text-Fig. 2).

4. The changes in the total fungal numbers roughly correspond with the changes observed in numbers and percentage occurrence of Aspergilli and Penicillia (Tables I and IV).

5. Although Fusaria were very rarely observed in the control soil dilutions, they were regularly encountered in the rhizosphere dilutions. Highest numbers and percentage occurrence was recorded in the case of pigeon-pea whereas it was almost absent in *Sorghum* (Table V; Text-Figs. 2 and 4).

6. *Macrophomina phaseoli* was not obtained in dilution plates. Highest percentage occurrence was recorded in the seedling stages of plants but in french bean and cluster bean a second increase was observed in the final estimation (Text-Fig. 4).

7. *Neocosmospora vasinfecta* was very rarely obtained in the dilution plates. Highest percentage was recorded on the roots of pigeon-pea.

Both *M. phaseoli* and *N. vasinfecta* were absent in the case of *Sorghum* (Text-Fig. 4).

TABLE V

Fusaria in the Rhizosphere

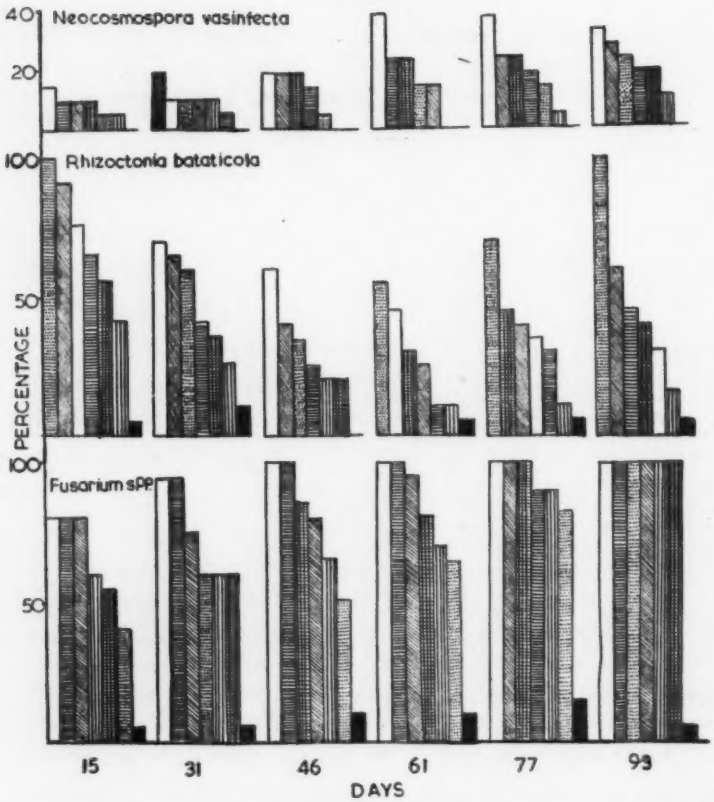
Plants	Thousands in 1 g. of moisture-free soil						Percentage occurrence in dilution plates						Percentage occurrence in root platings					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	61	77	93
Pigeon pea ..	40	42	58	38	15	20	8	10	10	8	5	7	80	95	100	100	100	100
Cluster bean ..	5	9	12	11	24	40	3	5	4	6	8	10	80	95	100	100	90	100
Cotton ..	4	18	18	15	13	11	3	4	5	3	4	5	60	60	65	70	90	100
Sorghum ..	0	0	1	2	1	1	0	0	0·23	0·5	0·3	0·37	5	5	10	10	15	5
Sesame ..	6	8	10	11	28	35	5	5	4	6	10	10	80	75	80	95	100	100
French been ..	15	9	20	34	5	60	5	3	5	8	10	10	40	60	50	65	80	100
Sann-hemp ..	2	26	20	13	19	20	5	7	5	6	5	4	55	60	85	80	100	100
Control soil ..	0	3	3	3	0	3	0	5	5	5	0	5	..	..	..	..	..	..

8. *Other Fungi.—Alternaria, Stemphylium, Curvularia, Helminthosporium, Trichoderma, Mucor, Rhizopus, Cunninghamella, Phoma, Diplodia, Chætomium*, etc., were often recorded in the rhizosphere dilutions and root plantings. In the unflowered plants the occurrence of these fungi was irregular but a definite increase in numbers and percentage occurrence was recorded in the final estimation of the flowered plants (Table IV; Text-Figs. 2 and 4).

DISCUSSION

Significantly greater accumulations of fungi, bacteria and actinomycetes were recorded in the rhizosphere than in the control soils (Tables I, II and III; Text-Fig. 1), confirming the observation reported by many of the previous workers (Starkey, 1929, 1931, 1938; Gräf, 1930; Timonin, 1940; Krassilnikov, 1945; Katznelson, 1946). Leguminous plants, namely, pigeon-pea, french bean, cluster bean and sann-hemp had highest numbers of bacteria in the rhizosphere than the non-legumes, an observation similar to that of Starkey (1931) in his studies on the influence of the development of higher plants upon the micro-organisms of the soil (Tables I, II and III; Text-Fig. 1).

Increase in bacterial numbers with the age of the plants was recorded. In the flowered plants the increase continued up to the time of flowering



TEXT-FIG. 4. Showing the percentage occurrence of *Rhizoctonia bataticola* (*Macrophomina phaseoli*), *Neocosmospora vasinfecta* and Fusaria in the root platings.
(Index same as in Text-Fig. 3)

followed by a fall during pod setting stages of plants, but in the unflowered plants the increase continued up to the final estimation (Table I; Text-Fig. 1). The behaviour of the fungal numbers on the other hand was different. The numbers in the flowered plants in general continued to decrease up to the time of flowering followed by an increase (Table I; Text-Fig. 1). The observed maximum ' rhizosphere effect ' during the active vegetative growth was also reported by Starkey (1938), Gräf (1930), Katznelson (1946), Krassilnikov (1945). Gräf (1930) in her studies on the rhizosphere microflora noted an increase in the total numbers of micro-organisms during the active growth of the plants followed by a decline during the ripening period

and a further decline after the harvest of the crop. The decrease in fungal numbers recorded prior to flowering was not reported by any of the previous workers, although Krassilnikov (1945) reported a gradual increase in the fungi after the maturity of the plant.

The actinomycete population of the soil was not much influenced by the roots in the early stages of plant growth but with the advance in age of the plant significant differences were obtained which may probably be due to the slow growth rate of actinomycetes (Tables I and III; Text-Fig. 1). Although the numbers of actinomycetes in the rhizosphere are greater than the fungi, the rhizosphere: soil ratios are not as much as fungi (Tables I and II). Despite the fact that the root effect is not so pronounced in fungi and actinomycetes, it is not improbable that their activity is as much modified as bacteria.

Rhizosphere effects with fungi have not attracted the same attention as those concerning bacteria and there are comparatively few records concerning the qualitative nature of the fungi (Thom and Humfeld, 1932; Timonin, 1940, 1941; Katznelson, Lochhead and Timonin, 1948). The percentage occurrence of Aspergilli in the rhizosphere and control soils was the same, but in the case of Penicillia lower percentage occurrence in the rhizosphere as compared to the control soil was observed (Text-Fig. 2). In general the changes in the total fungal numbers of the rhizosphere roughly correspond with the quantitative changes recorded in Aspergilli and Penicillia (Tables I, IV and V; Text-Figs. 1, 2 and 3).

Fusarium spp. also constituted one of the important genera in the rhizosphere fungal population. More than 50 per cent. of all the plant roots yielded *Fusarium* spp. except *Sorghum* (Table V; Text-Fig. 4). Although this fungus was abundant on the root surface comparatively lower numbers were obtained in the dilution plates (Table V; Text-Figs. 2 and 4).

Macrophomina phaseoli and *Neocosmospora vasinfecta* were not obtained in dilution plates. Highest percentage occurrence of the former was recorded during the seedling stages followed by a fall with the advance in age of the plants, while the percentage occurrence of the latter fungus increased with the age of the plant and the highest percentage was recorded in pigeon-pea on which it has recently been proved to be pathogenic by Sarojini (1952) (Text-Fig. 4). Similarly highest percentage occurrence of *Macrophomina phaseoli* was observed on the roots of french bean which has been proved to be most susceptible to this fungus (John Chinnayya, 1951). The predominance of this fungus on the root surface of plants in early stages of growth suggests that it may be a seedling pathogen.

The higher numbers of fungi found in estimations of flowered plants can be attributed to the preliminary stages of root colonization by soil saprophytes following decline in plant growth (Table IV; Text-Figs. 2 and 3). It may be that the common saprophytes of the soil find the root region uninhabitable as long as the plant growth is active but with the advent of senility they begin their invasion or colonization.

SUMMARY

1. Greater accumulations of fungi, bacteria and actinomycetes in the rhizosphere than in the control soils were recorded.

2. A decreasing order of 'rhizosphere effect' as follows was noticed: bacteria > fungi > actinomycetes.

3. Maximum 'rhizosphere effect' on bacteria was noticed during the time of flowering. Increase in fungal numbers after flowering was recorded.

4. Changes in the total fungal numbers correspond with the changes in numbers of Aspergilli and Penicillia, both in the dilution plates and the root platings.

5. The absence of certain predominant fungi like *Fusarium* spp., *Macrophomina phaseoli*, *Neocosmospora vasinfecta* in the rhizosphere dilutions emphasizes the necessity of assessing the physiological state in which they are extant in the rhizosphere.

6. *Sorghum* stands unique in that its rhizosphere had highest numbers of fungi among the non-legumes and highest numbers of actinomycetes among all the plants studied. The potential root pathogens so often encountered in the rhizosphere of other plants were absent in the case of *Sorghum*.

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PREVENTION OF PREMATURE BOLTING IN ONIONS FOLLOWING MALEIC HYDRAZIDE TREATMENT

By R. S. Choudhri, M.Sc., Ph.D. (Cornell), D.Sc.
(Professor of Agricultural Botany and Physiologist, College of Agriculture, Banaras Hindu University)
AND
V. B. Bhatnagar, M.Sc. (Ag.)
(Research Scholar)

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Introduction

Onions, botanically known as *Allium cepa* L., N.O. *Liliaceæ*, are commonly extensively grown in this part of the country in the month of November through December. Despite the common practice to raise seedlings in the nursery beds from viable seeds and subsequently transplanting them systematically in the field, it is not uncommon to raise them from the bulbs of the previous year, also known as sets. The distinct advantages of using sets in place of seeds and seedlings are well known. The sets can safely be planted considerably early (even in September) without waiting for the seeds to germinate and the seedlings to attain the proper size for transplantation. Planting can be done much more rapidly and the expense of growing the seedlings is obviated. The bulbs thus produced are usually larger in size and the crop matures a month or more earlier than when grown directly from seeds and seedlings.

Experience has shown that whereas onions raised from sets bolt to a large extent, even those raised from seeds exhibit on an average 25–30 per cent. or more bolting, at least in this part* of the country. The bulbs produced by the bolted plants are worthless as ripe onions. They are tough, exceedingly strong and will not keep. In view of the marked deteriorating effects on the culinary quality of onions, bolting is generally disliked by growers unless otherwise the plantation is done for raising seeds. Whether this behaviour owes its existence as an inherent character of certain seeds or is merely causal to prevailing environmental conditions cannot be said

* Banaras is situated in latitude 25° 19′ N. and longitude 83° 03′ E., at a height of 267 feet above mean sea-level.

with certainty unless detailed investigations are conducted on this aspect of the problem. The problem of premature bolting, however, remains. It was, therefore, considered useful to determine the effect of maleic hydrazide-1, 2-dihydropyridazine-3, 6 dione—a plant growth inhibitor and herbicide, reputed to solve many problems of this nature, on the bolting behaviour of onions.

Material and Methods

Maleic hydrazide (MH-30 formulation) was kindly supplied by the United States Rubber Company, Naugatuck, Chemical International Division, N.Y., as diethanolamine salt containing 30 per cent. active ingredient by weight and to whom the authors are greatly thankful.

Seeds of commonly grown Patna Red variety of onions were procured from Messrs. Suttons & Sons, seed growers, Calcutta, rigorously selected for uniformity of size and weight and all deformed and damaged seeds discarded. Seeds selected in this manner were sown in a well prepared nursery bed and when seedlings were approximately 4″ high, they were topped to avoid transpirational losses and transplanted in earthenware pots (9″×12″) filled with a well mixed half-and-half soil-manure mixture. Three seedlings were planted per pot and when fully established, they were thinned down to one in a manner that all the experimental pots contained single plants of uniform vigour. Watering and hoeing were carried on at regular intervals and four months later (one month before harvest) when plants were full grown and bulbs had undergone considerable development, they were segregated into seven experimental lots of two dozen plants each, six of these lots were used for treatment with maleic hydrazide and the seventh one to act as control. Two types of treatments, *viz.*, the treatment of tops and treatment of roots were planned for the experiment. Treatments to tops were administered in three different concentrations of 0·025, 0·10 and 0·25 per cent. through spraying, avoiding any run-off. Solutions of similar concentrations were also applied to three other lots through soil in the vicinity of the roots. Untreated controls were maintained side by side to clearly visualize, if any, changes accrued in the plants as a result of maleic hydrazide treatments.

Observations

At harvest time, the untreated control plants exhibited about 40 per cent. bolting, while there was a progressive suppression of bolting following top spraying as the concentration of maleic hydrazide increased from 0·025 to 0·25 per cent. (Table I).

TABLE I

Bolting Responses of Onion Plants following Top Spraying with Maleic Hydrazide; the Controls Exhibited on an Average 40 per cent. Bolting

Concentration (%) ..	0·025	0·10	0·25
Percentage bolts ..	16·5	8·0	Nil

As evident from the data presented in the table above, there was about 16 per cent. bolting following top spraying with 0·025 per cent. maleic hydrazide. Bolting was much less (only 8 per cent.) following 0·10 per cent. applications and none at all at a concentration of 0·25 per cent. (Plate I, Fig. 1).

Besides the above, the response of plants to treatments was also associated with other notable changes in their development. A progressive increase in the size of the bulbs was evident with the suppression effect on bolting (Plate I, Fig. 1), obviously because the flowering shoot, flowers and fruits are the largest consumers of food material, which in the absence of bolting may have been diverted into the bulbs for storage. It was further noticed that relatively low concentrations of maleic hydrazide, such as 0·025 per cent., which did allow a small percentage of plants to bolt were clearly characterized with delayed maturity. Thus, whereas the inflorescence of the untreated control plants entered a state of maturation, the flower initials in the case of the experimental plants treated with low concentrations of maleic hydrazide were rather immature (Plate II, Fig. 2).

Treatment of onion roots with maleic hydrazide solutions through soil was all the more effective (Table II).

TABLE II

Bolting Responses of Onion Plants following Root Treatment with Maleic Hydrazide; the Controls Exhibited on an Average 40 per cent. Bolting

Concentration (%) ..	0·025	0·10	0·25
Percentage bolts ..	Nil	Nil	Nil

There were hardly any cases where bolting occurred after the treatment. In a couple of cases where bolting shoots had started emergence before the maleic hydrazide treatment was administered, growth of such shoots stopped and they eventually dried and died within a few weeks following treatment (Plates I and II, Figs. 1 and 3).

Root treatment of onion plants was also associated with a better development of the bulbs on the one hand and with a larger production of roots themselves, on the other. All the same, these roots though larger in number were relatively short and attenuate (Plate I, Fig. 1). Better development of bulbs following these treatments may again be ascribed to better conditions of storage in the absence of bolting.

Treated onion plants had much broader leaves, though smaller in length in comparison to those of the untreated control plants (Plate I, Fig. 1), presumably because maleic hydrazide inhibits cell multiplication rather than cell elongation (Greulach and Atchison,[7] McIlrath,[14] Petersen,[16] and Zukel[20]).

Early maturity of the bulbs of plants treated with relatively high concentrations of maleic hydrazide, following both top and root applications as against delayed maturity of flowers on low concentration applications was also noted (Table III). This behaviour can be attributed to the fact that no new leaves were produced by the treated plants due to inhibition effect on the activity of the meristematic cells.

TABLE III

Certain After-Effects following Treatments of Tops and Roots of Onion Plants with Maleic Hydrazide in Different Concentrations

Particulars	Bulb maturation (days)*	Flower initiation (days)*	Seed formation (days)*
Control (untreated) ..	153	127	145
Top spraying with:			
0·025 MH	153	140	No seed formation till 153 days
0·10 MH	147	Nil	Nil
0·25 MH	140	Nil	Nil
Root treatment with:			
0·025 MH	149	Nil	Nil
0·10 MH	142	Nil	Nil
0·25 MH	135	Nil	Nil

* After planting.

DISCUSSION

Several points of interest are the outcome of the above enquiry. It has been noticed that maleic hydrazide treatments in certain concentrations prevented bolting in onions, either when sprayed onto the tops or when administered to the roots, indicating the bidirectional movement of maleic hydrazide. Beal,[2] and Ferri[6] using one or the other of the indole and the substituted phenoxy compounds have indicated the unidirectional transport of growth substances, while others like Hitchcock and Zimmerman,[8, 9] Skoog,[17] Mitchell and Brown,[15] and Choudhri[3] using the same compounds and in some cases their derivative salts and Linder[13] using maleic hydrazide record a bidirectional translocation of such substances. The true nature of translocation of the growth substances though controversial, more recent and comprehensive investigations confirm the latter view (Choudhri[3]). It is further clear that maleic hydrazide is capable of moving to long distances like other growth substances from the seat of application, thus, affecting the growth behaviour pattern of entire onion plants. Both when tops and roots were treated with adequate dosages of maleic hydrazide, the onion bulbs increased in size. This is explained on the basis of adequate food storage conditions in the absence of bolting.

Kosar and Thompson[12] were able to suppress bolting of lettuce and Hubbard and Rowland[10] that of spinach through maleic hydrazide applications. Similarly, White, *et al.*,[18] and Kennard, *et al.*,[11] pointed a delayed blossoming in the case of raspberries by 3–4 weeks on maleic hydrazide applications in 0·20 per cent. concentration. Barnard and Warden[1] and Crafts, *et al.*,[4] on the contrary, observed some stimulation effects on the bolting behaviour of lettuce when applied to young plants. Perhaps the age of the plants is an important factor in the determination of responses to such treatments.

A comparative study of the bolting behaviour of top and root treated onion plants indicates the consideration of another important point. Following top treatments with relatively low concentrations of maleic hydrazide, a small percentage of plants was observed to bolt. Contrariwise, there were no such cases of bolting following root treatments (*cf.* Tables I, II and III). Seemingly, therefore, maleic hydrazide was comparatively more readily absorbed by the roots than by the leaves. Working with tomato plants Zukel[19] reported a very slow entrance of maleic hydrazide through tops. He observed that only one-third of the solution entered the leaves after a lapse of 18 hours following application.

Of further interest, it was noted that the resultant responses were much more marked following root treatment of plants than tops, presumably because the terminal meristematic region, which grows to form the bolting shoot, is situated at the base of the bulb near the roots. Maleic hydrazide, therefore, seems to reach this point quicker than in case when top treatment is given.

It has also been noted in this investigation that the suppression of bolting was associated with increasing yields of bulbs. Maleic hydrazide is reported to inhibit mitosis, causing chromosome breaking (Darlington and McLeish[5]), and inhibiting cell division but not cell elongation (Greulach and Atchison,[7] McIlrath,[14] Petersen[16] and Zukel[20]). As a consequence of this, it seems reasonable to conclude that manufacture of food by the green leaves, subsequent to maleic hydrazide treatment, has not been affected and this supply in the absence of bolts, instead of being diverted to the growing shoot, takes a course towards the bulbs. Furthermore, the stored food in the bulbs, which is likely to be consumed, atleast partly, by the bolting shoots, when fresh manufacture by leaves due to advanced age of the plants receives a setback, remains unaffected for want of bolting. Causes of this nature, apparently, lead to the production of relatively large-sized bulbs following maleic hydrazide applications.

Conclusion

Maleic hydrazide treatments in suitable concentrations can advantageously be utilized to prevent premature and undesirable bolting in onions and thus help in increasing the yield and quality of bulbs.

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EXPLANATION OF PLATES

FIG. 1. Depicting certain effects following maleic hydrazide application on full grown onion plants.

Left.—Untreated (control) onion plant developing normal bolting shoot and having smaller bulb and relatively narrow leaves.

Centre.—Top-treated onion plant with 0·25% MH. Characteristic effects: absence of bolting, formation of large-sized bulb and broader leaves.

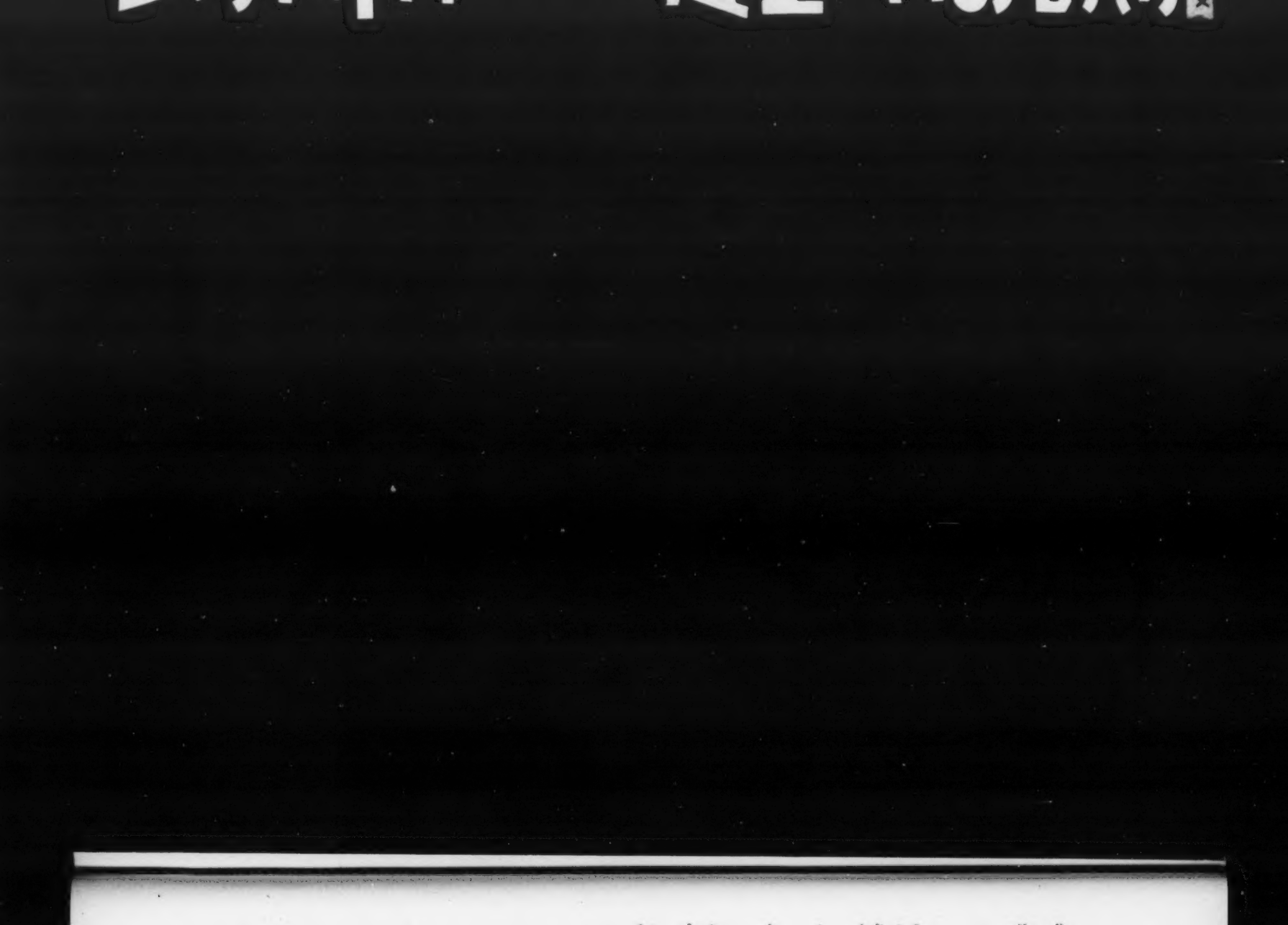

FIG. 1

FIG. 2

FIG. 3

II

Right.—Root-treated onion plant with 0·25% MH. Characteristic effects: Drying and dying of the bolting shoot which had developed prior to MH application, formation of larger bulb, broader leaves and larger number of attenuated roots.

FIG. 2. Top-spraying of full grown onion plants with low concentration of maleic hydrazide and its resultant effect on the maturation of flowers.

Left.—Inflorescence from untreated (control) plant detached after 153 days of plantation. Early maturity is obvious as the plants had entered the seed formation stage.

Right.—Inflorescence from 0·025% MH treated onion plants, depicting late maturity. Most of the flowers following this treatment, photographed after 153 days of plantation, are yet in full bloom and still others in bud state. No seed formation occurred at this age.

FIG. 3. A close up of the drying and dying scape (initiated before the treatment was administered) following root treatment of full-grown onion plants with 0·25% maleic hydrazide.

DETERMINATION OF NICOTINE IN TOBACCOS

By B. G. Chatterjee, B. Ramamoorthy, C. Dakshinamurti and K. C. Gulati

(Indian Agricultural Research Institute, New Delhi)

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I. Introduction

Nicotine is one of the important alkaloids of tobaccos and its content varies in different species within wide limits. Out of the several methods by which it can be extracted from a sample, the petroleum ether extraction[1] and the steam distillation[2] methods are by far the best. Extraction by these methods takes a minimum of a couple of hours and thus limits their application as a routine method.

Nicotine thus extracted as a free base is estimated either by the standard gravimetric method[3] or by physical methods such as colorimetric,[4] turbidimetric[5] and spectrographic.[6] Gravimetric method, though time consuming, gives accurate results only at higher concentrations of nicotine content whereas at lower values of the order of 10 to 1 p.p.m. the method cannot be recommended. Both colorimetric and turbidimetric methods depend upon the completion of a chemical reaction which at low concentration of nicotine content takes two to three hours and the reproducibility of results is also somewhat difficult. The spectrographic method is based on the principle of the characteristic absorption band of nicotine in the 2600 Å region. This is both accurate and expedient and is therefore preferable particularly in such breeds of low nicotine content as met with in the grafting experiments.

The present investigation was undertaken with a view to develop a suitable routine method for nicotine estimations in the plant breeding work and the manufacture of nicotine insecticides. Quick extraction from air-dried tobacco sample could be brought about by steam distillation under a slight positive pressure and the actual estimation has been carried out spectrographically. Both in the spectrographic as well as in the silico-tungstic acid methods, the nicotine estimated includes nor-nicotine as well. As nor-nicotine is usually present in tobaccos in traces, the estimated values are taken as the nicotine content. Preliminary results obtained have already been reported.[7]

II. Experimental Arrangements

Powdered and sampled tobacco is taken and nicotine is extracted by steam distillation method. In the standard A.O.A.C. method of extraction[2] usually 2 to 3 grams of sample is taken and distillate of 600 to 700 c.c. is collected for over an hour. The standard silicotungstic acid method was used for the estimation of nicotine.

The semi-micro method of distillation developed is a modification of Griffith and Jeffray's method[8] wherein soda lime is used instead of magnesium oxide. During the process of distillation with steam under positive pressure, fine magnesium oxide, used by Griffith, and Jeffray, is carried away into the condenser and this could be avoided by the use of soda lime instead. The results obtained using soda lime agreed very closely with those obtained by the standard and the magnesium oxide methods and for a sample studied the average values for these methods worked out to be 4·13%, 4·14% and 4·16% of nicotine on air-dried sample basis respectively.

Nicotine in the distillate is estimated by the spectrophotometric method using a Spekker Ultraviolet Photometer and a Hilger's Intermediate Quartz Spectrograph.

0·5 gram of powdered and sampled tobacco is transferred to the reaction tube and about half a gram of soda lime is added to it. The tube is electrically heated as to superheat the steam let in under positive pressure. Five to six minutes time was found sufficient to liberate all the nicotine present in the sample. The distillate is collected in N/20 hydrochloric acid and made upto 250 c.c. with acid of the same strength. An aliquot of the solution appropriately diluted to fall within the range of Beer's Law is taken for spectrographic analysis. The spectrum is recorded photographically over a range of densities from 0·0 to 1·0. If, however, the strength of the solution is approximately known the range could be reduced and the spectra of 4 samples could be recorded on a plate of $6\frac{1}{2}'' \times 4\frac{1}{4}''$ size. The exposures are taken at intervals of 0·025 units of density which can easily be read on the drum of the Spekker Photometer. The density corresponding to the match point at 2590 Å is noted and the nicotine content of the sample is calculated directly from a calibration curve drawn by taking several standard solutions. 2 cm. cells are used in the present investigation but for smaller concentrations of nicotine content cells upto 4 cm. length have been found necessary.

III. Results

The extinction coefficients were recorded directly at 2590 Å band head or concentration of nicotine varying between 1 to 15 p.p.m. Though Beer's

Law holds good upto a concentration of 30 p.p.m., the working range has always been found suitable to be within the range of concentrations studied.

The variation in the strength of hydrochloric acid has little effect on the absorption and the results obtained are as follows:—

TABLE I

Effect of Change in the Acid Strength on the Extinction Coefficient of Nicotine Solution of the Same Concentration

Normality of HCl	Extinction coefficient
0·2000	0·55
0·1000	0·56
0·0500	0·55
0·0250	0·55
0·0125	0·56

The nicotine extracted by the A.O.A.C. method is estimated by chemical and spectrographic methods for different samples and they are found to agree well within 2% variation. The results in Table II are the mean values of six sets of observations. The maximum error in both the methods is of the order of $\pm$ 2%.

TABLE II

Comparison of Chemical and Spectrographic Methods of Estimation of Nicotine in Solution

Nicotine in p.p.m.

Chemical	Spectrographic
89·0	90·5
87·1	85·3
80·3	81·0
54·5	55·0
46·8	48·4

Nicotine was extracted for different intervals of time by the rapid method of distillation developed and was estimated by the spectrographic method so as to find out the optimum time of distillation of all the nicotine present in a sample. The results are given in the figure.

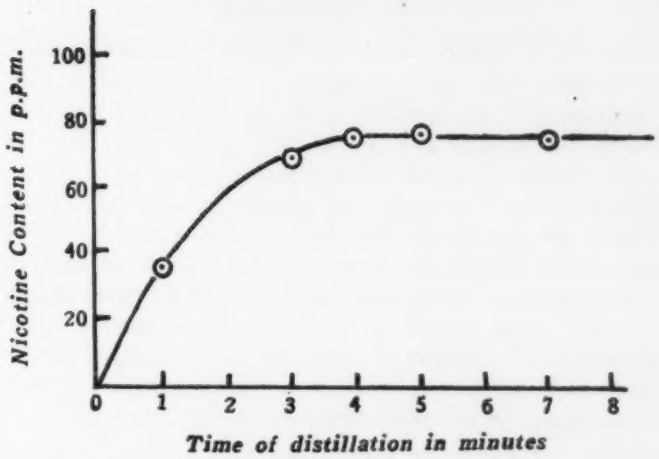

FIG. 1. Variation of Nicotine Extracted with Duration of Distillation.

It may be seen from the figure that almost all the nicotine can be extracted in about 4 minutes, as such extraction for about 6 minutes would ensure the complete extraction of the nicotine present in a sample within experimental limits.

Table III gives a comparison of the nicotine extracted by semi-micro rapid method and the standard A.O.A.C. method. The determinations are made spectrographically.

TABLE III

Comparison of A.O.A.C. and Semi-Micro Methods of Extraction

Nicotine content in p.p.m.

S. No.	A.O.A.C. method	Semi-micro rapid method
1	77·2	77·2
2	83·1	82·8
3	81·7	81·0
4	82·0	78·1
5	82·0	82·7
6	63·8	63·8
7	77·7	73·1
8	64·8	64·8

IV. CONCLUSION

Nor-nicotine is usually present in tobaccos, though in small quantities, and gets distilled along with nicotine. It is determined as nicotine both in the chemical and the spectrographic methods. The specific extinction coefficient of nor-nicotine however is 10% greater than that of nicotine. Hence it was preferred in the present investigation to take a distillate of representative tobacco and then work out the specific extinction coefficient rather than to use pure nicotine for that purpose as done by earlier workers. In all the samples studied, no special background correction was found necessary and perhaps this may be the characteristic feature of the Indian varieties. The close agreement between the results of A.O.A.C. and the semi-micro rapid methods of extraction and estimation permits the use of the method developed as a quick and reliable technique for the routine analysis of nicotine in nicotine insecticides and plant tissues.

V. SUMMARY

A convenient and rapid routine method for estimation of nicotine in tobaccos has been developed. The method is applicable to nicotine concentrations as low as 1 p.p.m. within $\pm$ 2% error. The method will have a special bearing for studying the biogenesis of nicotine in plant tissues.

Thanks are due to Mr. T. R. Srinivasan for verifying some of the results.

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ABNORMAL MITOSES IN TETRAPLOID YEASTS

By M. K. Subramaniam, F.A.Sc.

(Lecturer in Cytogenetics, Indian Institute of Science, Bangalore-3)

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Introduction

The unsatisfactory nature of the basic morphological criteria employed by yeast geneticists was elaborately discussed in a few recent contributions (Duraiswami and Subramaniam, 1950; Subramaniam, 1950 *a*, *b*, *c*; 1951) and it was indicated that for any ordered progress in the future a fruitful association with cytology is imperative. The demonstration of spontaneous and induced tetraploidy in yeasts (Subramaniam, 1945, 1947, 1951; Prema Bai and Subramaniam, 1947; Royan and Subramaniam, 1952) necessitated the belief that segregation in tetraploids if assumed as occurring in diploids—as has been done by Winge and Roberts (1950)—would appear really unique.

Winge (1951 *a*) states: "The fact is that a regular reduction of the chromosome complement does not occur in meiotic divisions of autotetraploids and, furthermore, that multipolar spindles lead to a random distribution of the chromosomes so that only rarely will well balanced cells arise." (p. 91). Roman, Hawthorne and Douglas (1951) in a paper which appeared a few months before that of Winge (1951 *a*) visualized the possibility that the exceptional ascus observed by them may have arisen from a tetraploid cell or that "the diploid spores may have been the consequence of an extra division in spore formation". They add: "It should be noted that the fusion of nuclei of like mating type following the extra mitosis would provide diploid mating clones from which tetraploid zygotes can be obtained" (p. 81). Winge (1951 *b*) admitted that Roman, *et al.* (1951), "had in their material one tetraploid ascus out of a total of 64 giving four diploid spores" (p. 236). While our cytological proof for an induction of polyploidy and the recovery of the diploid from such autotetraploids is brushed aside by Winge on the plea that autotetraploids cannot form normal spores, the results of Roman, *et al.*, are accepted even though they offered no cytological evidence. We would like to emphasize that the observations of Roman, *et al.*, are a confirmation of our earlier suggestion (Subramaniam and Krishna Murthy, 1949; Duraiswami and Subramaniam, 1950).

Lindegren and Lindegren (1951) following up the observations of Roman, *et al.* (1951), now report the existence of tetraploidy in *Saccharomyces*. They

admit a somatic doubling of chromosomes preceding spore formation (p. 890) and confirm our suggestion (Duraiswami and Subramaniam, 1950; Subramaniam, 1950 *a*, 1951) that diploid spores can develop parthenogenetically—a phenomenon denied by Winge (1935)—and that they can in turn form spores. Incidentally their paper also offers support for our claim that the difference between " polyhaploids " and " real haploids " is not merely one of " terminological interest " as claimed by Winge (1951 *a*, p. 90).

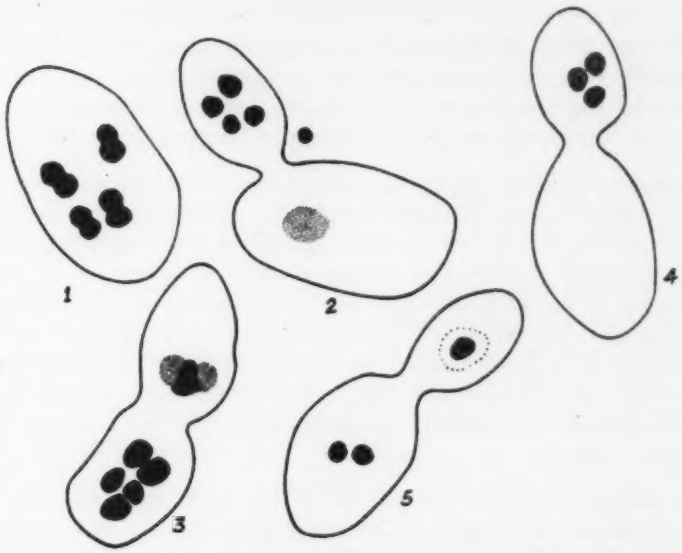
We stated (Subramaniam, 1950 *a*, 1951) that morphological criteria are valueless to differentiate between tetraploids, diploids and haploids. This is borne out by the following statement of Lindegren and Lindegren (1951). "Cultures 11294 and 11296 do not sporulate but the genotypes shown below may be inferred from the data obtained from 11295 and 11297" (p. 889). Winge (1935, 1951 *b*) asserted that his so-called " diploids " could be differentiated from the " haploids " by the ability of the former to form spores. When as reported by Lindegren and Lindegren (1951) tetraploids sporulate and some diploids do not, we presume that in their earlier work they have included diploids and polyploids under their so-called " diploids ".

The occurrence of polyploidy in yeasts furnishes the possibility of an array of genetic combinations ignored up till now by Winge and Lindegren. But that is not all. Even during vegetative divisions there are possibilities of the origin of new types by abnormal mitoses in tetraploids (*cf.* Vaarama, 1949) as was pointed out earlier by Ranganathan and Subramaniam (1948)

OBSERVATIONS

The technique of handling yeast for cytological investigations has already been dealt with elsewhere (Subramaniam, 1948 *a*; Duraiswami and Subramaniam, 1951). The distillery yeast described below was shown earlier to be a natural tetraploid (Ranganathan and Subramaniam, 1948). Plate III Photo 1 (Fig. 1) is the full metaphase of the distillery yeast from a Carnoy iron hæmatoxylin preparation. There are four pairs of chromatids. Cytokinesis in yeasts may or may not synchronize with karyokinesis (Subramaniam, 1946; Ranganathan and Subramaniam, 1948; Duraiswami and Subramaniam, 1951; Royan and Subramaniam, 1952). In Plate III Photo 2 (Fig. 2) the mother cell has a resting nucleus while the bud has four chromosomes. This is the normal behaviour. The chromosomes segregate into equal complements.

A considerable percentage of cells, however, show departures from this procedure. During anaphase the eight chromosomes may segregate into groups of five and three or six and two. In Plate III Photo 3 (Fig. 3) is illustrated a late anaphase from a Feulgen preparation in which the mother



cell has five chromosomes while the bud has only three. While cytokinesis synchronizes with karyokinesis in this instance, in Plate III Photo 4 (Fig. 4) it is absent and the bud alone shows three chromosomes. Since, as is evident in Plate III Photo 3 (Fig. 3) the chromosomes lie in different planes it has been found difficult to get a clear photograph showing a mother cell with six chromosomes and the bud with only two. But in Plate III Photo 5 (Fig. 5) is illustrated a normal mitosis of the diploid originating by such an abnormal mitosis. The mother cell shows two chromosomes, while the bud has a nucleus with a chromatin mass. The behaviour of the diploids, triploids, pentaploids and hexaploids originating by the abnormal mitoses of the tetraploid is elaborately discussed and illustrated elsewhere (Ranganathan and Subramaniam, 1952).

Discussion

The peculiar cytological behaviour of the distillery yeast described by Subramaniam and Ranganathan (1946) has been extensively discussed by Ranganathan and Subramaniam (1948). In that paper we remarked: "It has always been the custom to try to correlate and deduce abnormal behaviour of chromosomes in organisms, tissues or cells from normal patterns observed in innumerable plants and animals" (p. 389). Winge (1951 *a*) ignoring the latter publication selects a few disconnected statements and presents them as follows: "When cells were found with a large number of

bodies as, *e.g.*, 4, 6 and 8 " chromosomes " they believed that they represented the anaphase of the diploid, triploid and tetraploid nuclei although they actually bear little resemblance to a typical anaphase figure. They observed a mother cell with 5 " chromosomes " and its bud with 2 " chromosomes " and this was considered to be due to " lagging " (p. 90).

Lagging of chromosomes was described in yeasts by Levan (1947) and the cytological pictures presented by him bear a strong resemblance to the behaviour of the endopolyploid cells reported by Subramaniam (1948 *b*). Further, Skovsted (1948) from Winge's laboratory suggested chromosome lagging as the probable cause for the deviating yeast types observed after treatment with camphor. It is rather curious that while the above speculation of Skovsted, unsupported as it is by any cytological evidence, has met with the approval of Winge, exception should have been taken to our interpretation of observed abnormal cytological pictures as due to chromosome lagging.

It is difficult to understand Winge's emphasis on the shape and size of chromosomes in yeasts and on what he terms the " anaphase figure ". Chromosomes are defined not on the basis of their size or shape but as " the bodies into which the nucleus resolves itself at the beginning of mitosis and from which it is derived at the end of mitosis " (Darlington, 1932, p. 495). Similarly anaphase is described as " the stage at which the daughter chromosomes move apart in a nuclear division " (Darlington, 1932, p. 493). There is thus no emphasis on any specific type of configuration. We believe that the criteria on the basis of which we identified chromosomes in yeasts are much more acceptable than those of Winge. It would be well to remember that in *Drosophila*, all the chromosomes of a haploid set are not identical either in regard to their size or shape. Without trying the technique of others, Winge (1951 *a*) reproduces some photographs taken some fourteen years back. Not having repeated the experiments of others one can only presume that the views expressed are as old as the photographs themselves.

Lindegren and Lindegren (1951) after substantiating our claims regarding the varying grades of polyploidy remark that their controlled production of triploid and tetraploid yeasts " should not be taken as supporting Subramaniam's views on variations of ploidy " (p. 890). Lindegren's belated admission that triploid and tetraploid yeasts do occur is a tacit admission of the accuracy of our criticism—vigorously contested by Winge (1951 *a*, p. 90)—that since he and Winge analysed segregation in yeasts ignoring the possibility of polyploidy their work was based on a fundamentally erroneous assumption.

Summary

1. The occurrence of tetraploidy in yeasts reported by us several years back finds confirmation in some recent reports regarding the existence of tetraploid *Saccharomyces*. Tetraploidy furnishes the possibility of an array of genetic combinations ignored up till now by Winge and Lindegren.

2. Photomicrographs are presented to illustrate normal and abnormal mitoses in a tetraploid. The origin of diploids, triploids, pentaploids and hexaploids are described.

3. Abnormal mitoses common in tetraploid yeasts offer possibilities regarding the origin of new types. Lindegren's belated admission that triploid and tetraploid yeasts do occur is a tacit admission of the accuracy of our criticism that since he and Winge analysed segregation in yeasts ignoring the possibility of polyploidy, their work was based on a fundamentally erroneous assumption.

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EXPLANATION OF ILLUSTRATIONS

PHOTO 1. (FIG. 1). Full metaphase showing four pairs of chromatids. Carnoy. Iron Hæm. Length of cell, 6·1 μ.

PHOTO 2. (FIG. 2). Mother cell with a reconstituted nucleus and bud with four chromosomes. Carnoy. Iron Hæm. Length of mother cell, 6·1 μ.

PHOTO 3. (FIG. 3). Mother cell with five and bud with three chromosomes. Osmic Feulgen. Length of mother cell, 4·9 μ.

PHOTO 4. (FIG. 4). Bud showing three chromosomes at early telophase. Carnoy. Iron Hæm. Length of mother cell with bud, 8·4 μ.

PHOTO 5. (FIG. 5). Mother cell with two chromosomes at early telophase while the bud shows a reconstituted nucleus with a chromatin mass. Osmic Feulgen. Length of mother cell with bud, 8·2 μ.

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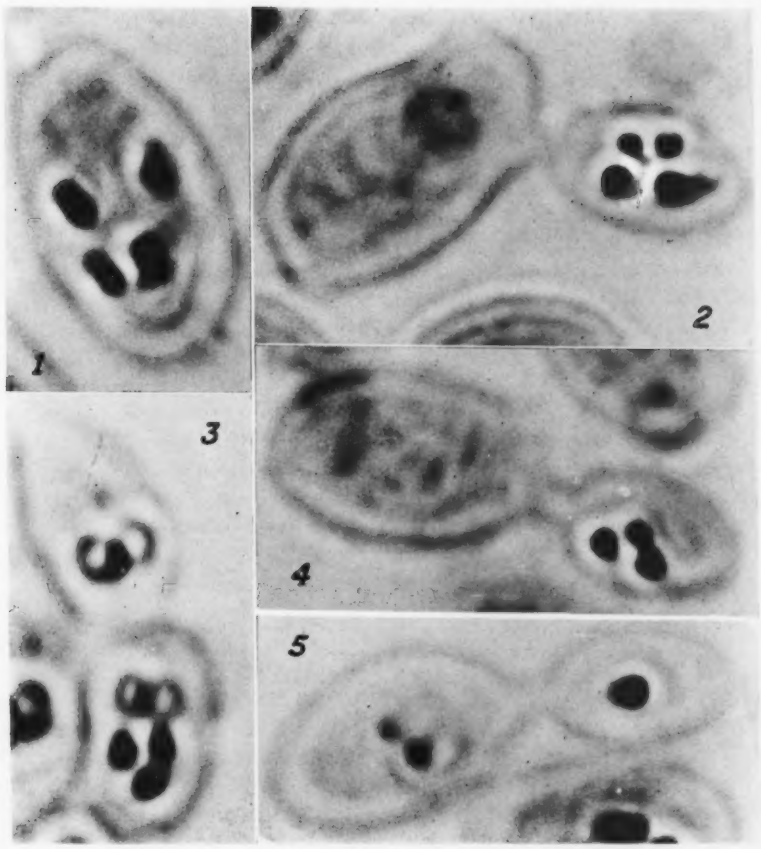
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